Final Scientific Report

Cover Page

BARD Project Number: IS-3836-06R

Date of Submission of the report: 22.9.2009

Project Title: Pre-natal Epigenetic Adaptation to Improve Thermotolerance Acquisition and Performance of Fast-Growing Meat-Type Chickens

Investigators	Institutions
Principal Investigator (PI): Shlomo Yahav	**ARO The Volcani Center**
Co-Principal Investigator (Co-PI): John Brake	**North Carolina State University**
Collaborating Investigators: Orna Halevy	**The Hebrew University of Jerusalem**

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Thermal manipulations, Embryogenesis, Thyroid and adrenal axis, Thermoregulation, Sensible heat loss, Satellite cells

Abbreviations commonly used in the report, in alphabetical order:

TM – thermal manipulation, T_b – body temperature, E- embryogenesis, RH – relative humidity, 24H – continuous TM, 12H – intermittent TM,

Budget: IS: $ 200,000 US: $ 110,000 Total: $ 310,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Appendix G6a

Appendix G6b

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	2		3	5
Submitted, in review, in preparation				
Invited review papers			1	1
Book chapters				
Books				
Master theses				
Ph.D. theses			1	
Abstracts	1	3	13	17
Not refereed (proceedings, reports, etc.)		5	6	11

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	2	1	4
Longer Visits (Sabbaticals)		1		1

Description Cooperation:

During the 1st year Shlomo Yahav visited John Brake's lab, studying techniques used there in order to be able to run similar incubation conditions in both the US and Israel. The 1st year was exploited to establish the optimal TM conditions using Cobb embryos in Israel and the Heritage strain in the USA. During the 2nd year, Shlomo Yahav spent a one-year sabbatical leave in John Brake's lab, where they both conducted several experiments to evaluate the improvement of thermotolerance and carcass quality of broilers on a semi-commercial scale, based on the 1st year findings and the on-going research in Yahav's lab (Manuscripts 1 and 2). This was in addition to the on-going experiments related to the 2nd year objectives of Dr. Brake. In parallel, Orna Halevy conducted several experiments in collaboration with Shlomo Yahav, to address the molecular and cellular mechanisms underlying the significant enhancement of the breast muscle growth and development in response to the embryonic TM. The 3rd year was spent for finalizing the studies on thermoregulatory and muscle development mechanisms and the evaluation of the carcass

quality improvement in Israel and US. John Brake then spent 2 weeks in Yahav's lab sharing his knowledge with Yahav's and Halevy's students.

Abstract: The necessity to improve broiler thermotolerance and performance led to the following hypothesis: **(a)** the thermoregulatory-response threshold for heat production can be altered by thermal manipulation (TM) during incubation so as to improve the acquisition of thermotolerance in the post-hatch broiler; and **(b)** TM during embryogenesis will improve myoblast proliferation during the embryonic and post-hatch periods with subsequent enhanced muscle growth and meat production**.**

The original objectives of this study were as follow: **1.** to assess the timing, temperature, duration, and turning frequency required for optimal TM during embryogenesis; **2.** to evaluate the effect of TM during embryogenesis on thermoregulation (heat production and heat dissipation) during four phases: (1) embryogenesis, (2) at hatch, (3) during growth, and (4) during heat challenge near marketing age; **3.** to investigate the stimulatory effect of thermotolerance on hormones that regulate thermogenesis and stress (T_4, T_3, corticosterone, glucagon); **4.** to determine the effect of TM on performance (BW gain, feed intake, feed efficiency, carcass yield, breast muscle yield) of broiler chickens; and **5.** to study the effect of TM during embryogenesis on skeletal muscle growth, including myoblast proliferation and fiber development, in the embryo and post-hatch chicks. This study has achieved all the original objectives. Only the plasma glucagon concentration (objective 3) was not measured as a result of technical obstacles.

Background to the topic: Rapid growth rate has presented broiler chickens with serious difficulties when called upon to efficiently thermoregulate in hot environmental conditions. Being homeotherms, birds are able to maintain their body temperature (T_b) within a narrow range. An increase in T_b above the regulated range, as a result of exposure to environmental conditions and/or excessive metabolic heat production that often characterize broiler chickens, may lead to a potentially lethal cascade of irreversible thermoregulatory events. Exposure to temperature fluctuations during the perinatal period has been shown to lead to epigenetic temperature adaptation. The mechanism for this adaptation was based on the assumption that environmental factors, especially ambient temperature, have a strong influence on the determination of the "set-point" for physiological control systems during "critical developmental phases." In order to sustain or even improve broiler performance, TM during the period of embryogenesis when satellite cell population normally expand should increase absolute pectoralis muscle weight in broilers post-hatch.

Major conclusions: Intermittent TM (39.5°C for 12 h/day) during embryogenesis when the thyroid and adrenal axis was developing and maturing (E7 to E16 inclusive) had a long lasting thermoregulatory effect that improved thermotolerance of broiler chickens exposed to acute

thermal stress at market age by lowering their functional T_b set point, thus lowering metabolic rate at hatch, improving sensible heat loss, and significantly decreasing the level of stress.
Increased machine ventilation rate was required during TM so as to supply the oxygen required for the periods of increased embryonic development.
Enhancing embryonic development was found to be accomplished by a combination of pre-incubation heating of embryos for 12 h at 30°C, followed by increasing incubation temperature to 38°C during the first 3 days of incubation. It was further facilitated by increasing turning frequency of the eggs to 48 or 96 times daily.
TM during critical phases of muscle development in the late-term chick embryo (E16 to E18) for 3 or 6 hours (39.5°C) had an immediate stimulatory effect on myoblast proliferation that lasted for up to two weeks post-hatch; this was followed by increased hypertrophy at later ages. The various incubation temperatures and TM durations focused on the fine-tuning of muscle development and growth processes during late-term embryogenesis as well as in post-hatch chickens.

Scientific and agriculture implications: This study proved for the first time **that TM during broiler embryogenesis has** the ability to establish: **a.** a long lasting alteration in the thermoregulatory threshold response which, no doubt, improved thermotolerance of broilers; **b.** the capacity to improve performance by using TM.
The agriculture implication lies within the adoption of these procedures in commercial hatcheries.

Achievements and innovations: This study demonstrated for the first time that utilizing the embryogenesis (E) period of broilers, in particularly E7 to E16 as the “critical phase” for TM of chick embryos, significantly enhanced thermotolerance. A continuous (as negative control) or intermittent (12 h/day – an optimal treatment) TM at 39.5°C and 65% RH during the "critical period" were chosen from several prior treatments that had been used in order to find the optimal treatment. During TM, egg shell temperature, heart rate, and oxygen consumption were elevated as embryos were in their ectothermal phase, but from the end of the TM untill hatching these variables were significantly lower than in the control, in both treatments. Moreover, plasma concentrations of the thyroid hormones were significantly lower in the two treatments during and after TM, until hatching. Plasma corticosterone concentration of the TM-treated embryos was significantly lower after the TM but significantly higher at hatching. These data led to the conclusion that TM during the period of thyroid and adrenal axis

development lowered their functional set point, thus lowering metabolic rate at hatching and improving thermotolerance. However, the continuous TM (24H) caused a significant decline in hatchability coupled with significantly lower BW and body temperature (T_b) at hatching. The intermittent-treated (12H) chicks showed similar results as the controls, but had significantly lower T_b. Subsequent thermal challenge at marketing age demonstrated a significant improvement in thermotolerance of both the 12H and 24H TM treated broilers, which was characterized by a significantly lower level of stress evidenced by the level of plasma corticosterone, and rate of mortality.

It was also found during the course of these studies that an increased machine ventilation rate was required during TM so as to supply oxygen required for the periods of increased embryonic development. Enhancing embryonic development during early incubation was found to lead to a slightly advanced embryo from E7 to E16. A more advanced embryo could produce less heat during TM if the yolk sac was smaller. This was accomplished in these studies by a combination of pre-incubation heating at 30°C for 12 h followed by incubation at 38.0°C for the first 3 days of incubation. Early development was further enhanced by increasing the turning frequency to 48 or 96 times daily as this also appeared to stimulate early embryogenesis.

It can be concluded that TM during the portion of embryogenesis when the thyroid and adrenal axis developed and matured has a long lasting effect that improved thermotolerance acquisition of broiler chickens. Whereas intermittent TM had no significant effect on hatchability and performance parameters, continuous TM negatively affected these parameters. Therefore, we proposed that the intermittent TM will be the one to be used commercially.

The main innovation of this study lies in accumulating evidence showing that in broilers, the epigenetic adaptation approach, associated with changes in the incubation environment with emphasis on fine-tuning of the level and duration of stress to coincide with the “critical phase,” can elicit efficient long lasting thermoregulatory adaptation.

The cellular and molecular basis of promoting muscle development and growth by TM during late-term chick development was explored under Objectives 4 and 5. Preliminary studies led us to conduct the TM at 39.5ºC from embryonic days 16 to 18 (E16 to E18) for 3h or 6 h daily (3H and 6H, respectively). The results showed that TM augments hypertrophy as of 13 days of broiler age, as manifested by higher diameter of myofibers and greater absolute muscle growth relative to controls, until 35 days of age. TM had immediate (as of E17) and later (up to 2 weeks posthatch) effects on elevating muscle cell proliferation. This was indicated by higher DNA incorporation of thymidine and a higher number of cells expressing PCNA in intact

muscle, accompanied by higher Pax7 levels—all reflecting a higher number of myogenic cells, and suggesting that the increased hypertrophy can be attributed to a higher reservoir of myogenic progeny cells produced in response to the TM. Whereas hypertrophy was similar in both TM groups, cell proliferation and Pax7 levels were more robust in the 6H muscle, mainly post-hatch, suggesting a differential effect of various TM periods on cell reservoir vs. hypertrophy, and a high sensitivity of myoblasts to relatively small changes in heat duration with respect to these processes, which was manifested both in the short and long term. IGF-I levels were higher in the muscle of the TM groups than in that of controls, implying a mechanism by which heat manipulations in chicks affected muscle development, with locally secreted IGF-I playing a major role.

TM during incubation was found to significantly improve the relative weight of breast muscle coupled with a significant decline in relative abdominal fat pad weight and feed conversion.

Agriculture and or economic impacts of the research findings: Mortality and morbidity significantly increase from late spring to autumn as a result of heat spells. TM in commercial hatcheries can be used for two main purposes: **1.** to improve thermotolerance acquisition and by that to reduce mortality from heat spells, mainly during the latter period of growth. Mortality rate during this period is the highest, and the main cause for economic losses. **2.** to improve broiler carcass quality (breast muscle, abdominal fat pad). Improving relative breast muscle weight by approximately 1% may contribute million(s) of US$ to the industry in both countries.

Details of cooperation: During the 1st year Shlomo Yahav visited John Brake's lab, studying techniques used there in order to be able to run similar incubation conditions in both the US and Israel. The 1st year was exploited to establish the optimal TM conditions using Cobb embryos in Israel and the Heritage strain in the USA.

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The 3rd year was spent for finalizing the studies on thermoregulatory and muscle development mechanisms and the evaluation of the carcass quality improvement in Israel and US. John

Brake then spent 2 weeks in Yahav's lab sharing his knowledge with Yahav's and Halevy's students.

List of publications:

Piestun, Y., Shinder, D., Ruzal, M., Halevy, O., Brake, J. and Yahav, S. (2008). Thermal manipulations during broiler embryogenesis: effect on the acquisition of thermotolerance. ***Poult. Sci.*** **87:1516-1525.**

Piestun, Y., Shinder, D., Halevy, O. and Yahav, S. (2008). The effect of thermal manipulations during development of the thyroid and adrenal axes on in-hatch and post-hatch thermoregulation**.** *Thermal Biol.* 33:413-418.

Piestun, P., Harel, M., Barak, M., Yahav, S. and Halevy, O. (2009). Thermal manipulations in late-term chick embryos affect skeletal muscle development: immediate and long-term effects on myoblast proliferation and muscle hypertrophy. *J. Appl. Physiol.* 109:233-240.

Leksrisompong, N., Romero-Sanchez, H., Plumstead, P.W., Brannan, K.E., Yahav, S. and Brake, J. (2009). Broiler incubation. 2. interaction of incubation and brooding temperatures on broiler chick feed consumption and growth. *Poult. Sci.* 88: 1321-1329.

Piestun, Y., Halevy, O. and Yahav, S. (2009). Thermal manipulations of broiler embryos – the effect on thermoregulation and development during embryogenesis. *Poult. Sci.* (in press).

Yahav, S. (2009). Alleviating heat stress in domestic fowl – different strategies. *Worlds Poult. Sci. J.* 65 (4): (in press). (Review).

Appendix

Published papers

Papers in press